

[ZIRAM]

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MetabolismStudy(85-1)

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DATA EVALUATION REPORT

ZIRAM

Study Type: METABOLISM ❏ RAT (85-1)

Prepared for

Health Effects Division
Office of Pesticides Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

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*Managed by Lockheed Martin Energy Systems, Inc., for the U.S. Department of Energy under Contract No. DE-AC05-84OR21400

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[ZIRAM]

Metabolism Study (85-1)

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Date: _____

DATA EVALUATION REPORT

STUDY TYPE: Metabolism ☒ Rat (85-1)

TOX. CHEM. NO: 931

P.C.CODE.: 034805

MRID NO.: 423910-01

TEST MATERIAL: Ziram

SYNONYMS: Bis(dimethylcarbomodithioato-S,S')zinc; bis(dimethyldithiocarbamate)zinc; zinc dimethylcarbamate; dimethyldithiocarbamic zinc salt; zinc bis(dimethylthiocarbamoyl) disulfide; methyl cymate; Methasan; Zimate; Zirbeck; Karbam White; Corozate; Fuclasin; Fuklasin; Zerlate

STUDY NUMBER: HLA 6225-106

SPONSOR: Ziram Task Force, c/o UCB Chemicals Corporation, Norfolk, VA

TESTING FACILITY: Hazleton Laboratories America, Inc., 3301 Kinsam Boulevard, Madison, WI 53704

TITLE OF REPORT: Metabolism of Ziram in Rats

AUTHOR: Theresa Cheng

STUDY COMPLETION DATE: February 1, 1991

REPORT ISSUED: July 9, 1992

EXECUTIVE SUMMARY: Groups of 15 male and 15 female rats were administered Ziram/¹⁴C-Ziram by gavage at doses of 15 mg/kg (Group 2, single low dose), 15 mg/kg/day for 14 days followed by a single dose of radiolabeled Ziram (Group 3), or 352 mg/kg (Group 4, single high dose). Controls (Group 1) received only the methylcellulose vehicle. Radioactivity excreted in the urine and feces was monitored for 168 hours (single low dose, multiple low dose, and single high-dose), and expired air for all three dose groups was monitored for 96 hours. Additionally, radioactivity in tissues and carcass were measured.

Clinical signs were limited to excessive salivation, lacrimation, rough hair coat, and white matter in the feces for several animals in dose Group 2 and 3. Overall recovery of administered radioactivity ranged from 78.9% to 92.4%. ¹⁴C-Ziram derived radioactivity was excreted in the expired air, feces, and urine. There were no significant quantitative or temporal differences in excretion of radioactivity between males and females. For all three treatment groups, excretion of ¹⁴C-Ziram derived radioactivity (average for both sexes) was greatest in expired air (37%, 41%, and 50% for Groups 2, 3, and 4, respectively), and was associated with both CO₂ and volatile fractions. Urinary excretion accounted for 17 to 35% of the administered

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radioactivity and was slightly greater in the multiple low-dose group. Fecal excretion accounted for 9 to 18% of the administered radioactivity and was similar for all dose groups. Percent of radioactivity administered was low in tissues (<1%) and carcasses ($\leq 1\%$) in all dose groups. Time-course data for excretion of ^{14}C -Ziram indicated rapid excretion via expired air in both low-dose groups (<24 hours) and in <48 hours in the high-dose group. Urinary and fecal excretion was nearly complete within 72 hours for both low-dose groups, but appeared to be multiphasic in the high-dose group with excretion peaks at 0-8 hours, 24-72 hours, and at 96 hours.

Orally administered Ziram appears to be rapidly absorbed and excreted via the urine and expired air, and significant amounts are excreted in the feces. Small amounts are widely distributed in the body.

Classification: Core Supplementary

This study satisfies, in part, the guideline requirements for a metabolism study (85-1) in rats. This study was intended to provide only information on the absorption, distribution and excretion of ^{14}C -Ziram.

Special Review Criteria (40 CFR 154.7) None

A. MATERIALS

1. Test material: Ziram

Description	Unlabeled test material	Radiolabeled test material ^{14}C -Ziram
	crystalline solid	crystalline solid
Purity (determined by TLC, GC, or HPLC/UV)	>99%	96.9%
Specific activity	NA	13.9 mCi/mol
Position of radiolabel	NA	tertiary thioamine

NA = not applicable

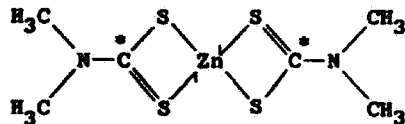
Lot No.: 027F9221 (^{14}C -Ziram); G3463 (nonradiolabeled Ziram)

Purity: 96.9% (^{14}C -Ziram); >99% (nonlabeled Ziram)

Stability of compound: stable in carboxymethylcellulose vehicle for duration of dosing period (14 days)

CAS No.: 137-30-4

Structure:



*Position of radiolabel

2. Vehicle and/or positive control

The vehicle was carboxymethylcellulose. No positive control was used.

3. Test animals

Species: rat
Strain: Sprague-Dawley CrI:CDR (SD)BR
Age and weight at study initiation: 6-10 weeks; 150-200 g
Source: Charles River Laboratories, Portage, MI
Housing: Individually in metabolism cages

Environmental conditions:

Temperature: $72 \pm 3^{\circ}\text{F}$
Humidity: $50 \pm 20\%$
Air changes: not provided
Photoperiod: 12 hr light/dark
Acclimation period: 8 or 9 days

4. Preparation of Dosing Suspensions

For treatment Groups 2 and 3, 66-67 mg of nonradiolabeled Ziram and 9-10 mg ^{14}C -Ziram were mixed with ≈ 40 ml of 0.5% carboxymethylcellulose (CMC) solution. For Group 4, the dosing solution was prepared by mixing 3.74 g of nonradiolabeled Ziram and 9.1 mg of ^{14}C -Ziram in 40 ml of 0.3% CMC. All dosing suspensions were mixed overnight prior to dosing. The concentration of the dose aliquots was verified by radioassay.

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B. STUDY DESIGN

The experiments described in the study report (MRID No. 423910-01) were designed to quantitate the absorption, distribution and excretion of ¹⁴C-Ziram in male and female rats following administration. Rats were randomly assigned to treatment and control groups as shown in Table 1.

Group No./Treatment	Dose ^a (mg/kg)	Route of administration	No. of animals		Time of sacrifice ^b
			Males	Females	
1 Control ^c	0	oral	2	2	
2 Single low dose	15	oral	5	5	7 days
3 Multiple low dose ^d	15	oral	5	5	7 days
4 Single high dose ^e	352	oral	5	5	7 days

Data taken from pp. 13 and 24, MRID No. 423910-01

^aDosing volumes were 2 ml for all groups except group 4 that received 1 ml.

^bDays following final ¹⁴C-Ziram dose

^cControls received 0.5% carboxymethylcellulose

^dFourteen daily doses of nonradiolabeled Ziram (adjusted for body weight on days 8-14) followed by a single dose of ¹⁴C-Ziram on day 15.

^eHigh dose was originally 750 mg/kg but downwardly adjusted due to aspiration of test material resulting from the high volume required for 750 mg/kg dose.

Signed and dated GLP (02/22/91) and quality assurance statements (02/01/91) were present.

C. METHODS**1. Dosing**

Dose volumes were 1-2 ml.

2. Rationale for Dose Selection

No rationale for dose selection was provided.

3. Collection and Preparation of Samples

The following samples were collected for analysis. All samples except whole blood were stored at <0°C until analyzed. Whole blood samples were refrigerated (temperature not specified). All solvents were HPLC grade or equivalent.

- Urine ☒ Urine samples from Groups 2, 3, and 4 were collected at 0-6, 6-12, and 12-24 hours after the radiolabeled dose and daily thereafter for 7 days. Samples were collected in a plastic container (immersed in an ice bath) and weighed.
- Feces ☒ Fecal samples from Groups 2, 3, and 4 were collected at 0-6, 6-12, and 12-24 hours after the radiolabeled dose and daily thereafter for 7 days. Fecal samples were collected in plastic containers surrounded by ice, frozen in liquid nitrogen, powdered, and duplicate aliquots weighed and combusted.

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- d. Expired air ❏ Expired CO₂ was trapped with ethanolamine:ethoxyethanol (1:3), and organic volatiles were trapped by activated carbon in glass tubing. Samples were collected in both traps at 0-4, 4-8, 8-12, and 12-24 hours after administration of the radiolabeled Ziram, and daily thereafter for 4 days. Analysis was conducted in animals from Groups 2, 3, and 4.
- e. Tissues/organs/carcass ❏ Fifteen matrices were analyzed for radioactivity and included: blood, fat, liver, bone (femur), pituitary gland, thyroid gland, ovaries, testes, heart, lungs, kidneys, muscle (thigh), spleen, uterus, and carcass. Blood (≈ 2-5 ml) was collected in heparinized tubes, mixed to assure homogeneity, and duplicate aliquots (0.2 g) weighed for combustion. Fat samples were digested with Carbo SorbR for 2 days and duplicate aliquots weighed and analyzed by liquid scintillation counting (LSC). Carcasses were ground, homogenized, and duplicate aliquots weighed and combusted. Other tissues were homogenized and duplicate aliquots weighed for combustion.
- f. Cage wash ❏ Following collection of fecal and urine samples, the cages were washed with 1% trisodium phosphate. Weights of the trisodium phosphate solutions used for washing were recorded.
4. Radioanalysis and calculations

LSC was performed for 5 minutes or 100,000 counts. CPM were automatically converted to dpm using an external standardization technique and an instrument-stored quench curve (derived from sealed, quenched standards). Validation of radioanalysis procedures was conducted by analyzing triplicate ¹⁴C-Ziram-spiked samples of urine, feces, blood, and liver from control animals. Recovery of added radioactivity was >95% and, therefore, measured dpm were not corrected for recovery. The following calculations were used:

Radioactivity in samples (¹⁴C dpm/g sample):

$$\text{dpm/g} = \frac{^{14}\text{C dpm in sample aliquot}}{\text{aliquot weight (g)}}$$

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Radioactivity in tissues (ug equivalents ^{14}C -Ziram/g tissue): _____

$$\mu\text{g equivalents/g} = \frac{\text{dpm/g}}{\text{specific activity (dpm}/\mu\text{g})}$$

Percent of total dose/sample: _____

$$\text{Percent total dose} = \frac{\text{sample } ^{14}\text{C (dpm/g)} \times \text{sample weight(g)}}{\text{total } ^{14}\text{C dose (dpm)}} \times 100$$

5. Fractionation and identification of metabolites _____

Characterization of metabolites was not a part of the protocol for this study.

6. Kinetics

Derivation of time-course values ($t_{1/2}$, k_d , V_d etc.) were not included in the study protocol.

6. Statistical analysis

Variability about the mean (e.g. variance, standard deviation) were the only statistical data derived.

D. RESULTS

1. Toxicity

There were no deaths that could be attributed to the test material (a Group 3 male was found dead at 96 hours but cause of death was attributed to suffocation resulting from CO_2 accumulation in the test chamber). Clinical signs including excessive salivation (one female) and rough hair coat (5 females) were observed at various time points for Group 3. In Group 4, excessive salivation (2 males), lacrimation (1 male), rough hair coat (3 females), white matter in the feces (3 females, 3 males), dark urine (1 male), and white material possibly from the urine (3 males) were noted. The toxicologic relevance of these observations and their association with the treatment are unclear.

2. Recovery of radioactivity

Mass balance data for administered ^{14}C -Ziram are shown in Table 2. Total recovery of ^{14}C -Ziram-derived radioactivity ranged from 78.9% to 92.38%. Over the total collection period, most of the administered radioactivity in all three dose groups was

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TABLE 2. TOTAL ¹⁴ C RECOVERY (%)							
Sex	Urine *	Feces	Tissues	Carcass	Volatiles	CO ₂	Total
Group 2 - Single Low Dose (15 mg/kg)							
Male	26.34±1.4	18.43±1.4	0.75±0.04	0.89±0.05	15.76±4.5	21.70±2.4	83.86±3.3
Female	25.46±2.4	15.29±4.8	0.89±0.1	0.99±0.1	15.61±3.7	20.70±3.1	78.93±3.8
Group 3 - Multiple Low Dose (15 mg/kg)							
Male	32.60±8.4	12.50±8.3	0.68±0.08	1.00±0.08	13.83±2.8	26.20±2.5	86.81±2.2
Female	35.41±7.7	12.24±4.3	0.81±0.1	1.11±0.1	20.63±9.3	22.18±2.7	92.38±9.9
Group 4 - Single High Dose (352 mg/kg)							
Male	22.61±5.1	9.03±3.7	0.41±0.1	0.70±0.08	30.53±7.4	22.68±4.0	85.97±4.7
Female	17.46±6.9	18.25±4.5	0.52±0.07	0.73±0.03	28.30±7.6	19.28±2.5	84.55±3.3

Data taken from Tables 4-6, pp. 27-32, MRID No. 423910-01.

*Includes cage wash and cage wipe.

excreted in expired air, urine, and feces. Relatively small amounts (generally <1%) remained in the tissues or carcass. For all three dose groups, the greatest amount of tissue radioactivity was found in the blood and liver (Table 3). Radioactivity in the other tissues examined was generally <0.01% and always <0.06%. There did not appear to be biologically significant sex-related differences in excretion, distribution, or retention of ¹⁴C-Ziram-derived radioactivity in any of the dose groups. On a ug equivalent/g tissue basis, the greatest concentration of ¹⁴C-Ziram-derived radioactivity was consistently found in the blood and highly perfused organs (liver, kidney, lungs, heart, thyroid and spleen).

TABLE 3. DISTRIBUTION (ug EQUIVALENTS/g) OF ¹⁴ C-ZIRAM DERIVED RADIOACTIVITY IN RATS FOLLOWING ORAL ADMINISTRATION							
	Blood	Liver	Lungs	Heart	Kidneys	Spleen	Thyroid
Group 2 - Single Low Dose (15 mg/kg)							
Males	1.335	1.216	0.403	0.596	0.657	0.326	0.342
Females	2.184	1.156	0.582	0.719	0.875	0.471	0.481
Group 3 - Multiple Low Dose (15 mg/kg)							
Males	2.101	1.270	0.632	0.462	0.788	0.605	0.564
Females	2.469	1.085	0.720	0.681	0.892	0.813	0.586
Group 4 - Single High Dose (352 mg/kg)							
Males	32.096	9.241	6.464	5.876	8.239	7.656	4.527
Females	41.382	10.151	10.317	7.866	11.030	10.056	7.310

Data taken from Tables 7-9, pp.33-38, MRID No. 423910-01.

6. Kinetics

Quantitative analysis ($t_{1/2}$, k_{el} , V_d , etc.) of time-course data were not conducted. However, graphic representation of elimination data were provided and showed that the majority of administered radioactivity by all routes (feces, urine, expired air) was eliminated by 24 hours and was nearly complete at 72 hours for both the single low-dose group and multiple low-dose group (Groups 2 and 3). For the single low dose group, a slight increase in radioactivity in the feces at 144-168 hours suggests possible biphasic fecal excretion. Curiously, this was not observed for the multiple low-dose group but was indicated at 96 hours in the single high-dose group. Elimination of administered radioactivity in expired air appeared to be biphasic for both males and females in the high-dose group exhibiting an initial elimination at 0-8 hours followed by a rapidly initiated and continuous elimination from 12 to 48 hours and rapid decline to 72 hours. Fecal excretion of radioactivity from high-dose rats exhibited a triphasic pattern with peak excretion at 0-12 hours, 24-72 hours, and at 96 hours. Urinary excretion was similar but did not exhibit the elimination peak at 96 hours. There did not appear to be any biologically relevant or statistically significant differences in excretion between males and females.

E. DISCUSSION

Groups of 15 male and 15 female rats were administered Ziram/ ^{14}C -Ziram at doses of 15 mg/kg (single dose), 15 mg/kg/day for 14 days followed by a single dose of radiolabeled Ziram, or 352 mg/kg (single dose). Radioactivity excreted in the urine and feces was monitored for 168 hours (single low dose, multiple low dose, and single high-dose), and expired air for all three dose groups was monitored for 96 hours. Additionally, radioactivity in tissues and carcass were measured.

Clinical signs were limited to several animals in dose Groups 3 (multiple low dose) and 4 (single high dose), and characterized as excessive salivation, lacrimation, rough hair coat, and white matter in the feces. Overall recovery of administered radioactivity ranged from 78.9% to 92.4%. ^{14}C -Ziram derived radioactivity was excreted in the expired air, feces, and urine. With the exception of the fecal excretion in the high-dose group, there was no substantial sex-dependent variability in radioactivity excreted in the various matrices. The low percentage of radioactivity in the feces of high-dose males could be attributed to the homogeneity problem described by the study author; presence of feed in the fecal sample and the "nature of Ziram". Expired radioactivity was associated with both CO_2 and volatile fractions and the percentage excreted was similar among the three treatment groups. For all three treatment groups, excretion of ^{14}C -Ziram-derived radioactivity (average for males and females combined) was greatest in expired air (37%, 41%, and 50% for Groups 2, 3, and 4, respectively, for combined CO_2 and volatile fractions). Urinary excretion accounted for 17 to 35% of the administered radioactivity and was slightly greater in the multiple low-dose group. Fecal excretion accounted for 9 to 18% of the administered radioactivity. Relative to the total radioactivity administered, both tissue burden (<1%) and carcass ($\leq 1\%$) radioactivity remained very low for all dose groups. Time-course data for excretion of ^{14}C -Ziram indicated rapid excretion via expired air in both low-dose groups (<24 hours) and in the high-dose group (<48 hours). Urinary and fecal excretion was nearly complete within 72 hours for both low-dose groups, but appeared to be multiphasic in the high-dose group with excretion peaks at 0-8 hours, 24-72 hours, and at 96 hours.

The unaccounted for radioactivity notwithstanding, orally administered Ziram appears to be rapidly absorbed and excreted via expired air and urine. Small amounts of absorbed ^{14}C are widely distributed in the body with the greatest concentrations appearing in well perfused organs. The current study did not provide data (nor was it intended to) for assessing metabolite formation. Therefore, it is not possible to

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determine if fecal radioactivity represents unabsorbed parent compound, metabolites formed in the lumen of the gastrointestinal tract, or biliary metabolites.

F. STUDY DEFICIENCIES

1. Major

Mass balance was adequate for guidelines but of marginal adequacy for a well-conducted absorption-distribution-excretion study. Although the loss of radioactivity via exhaled volatiles is feasible, it is speculative in view of the available data. Rationale for inconsistent recovery of fecal radioactivity requires more detailed explanation. That variability in measurement of fecal radioactivity was due to lack of homogeneity and the "nature of Ziram" is vague.

2. Minor

Data tabulations lack indicators of variability around the mean, and graphic representations lack error bars making an accurate assessment of real differences difficult.